

FIG.1A

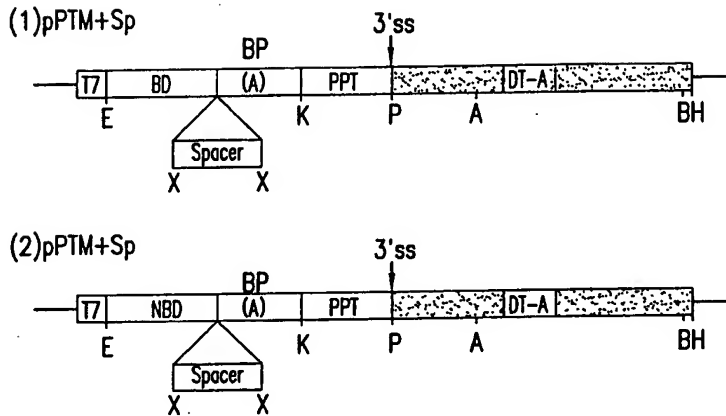


FIG.1B

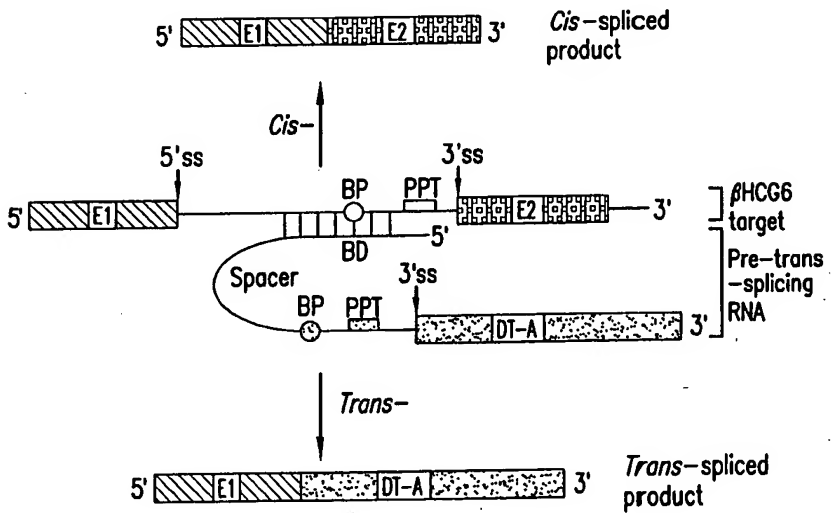


FIG.1C

Schematic drawings of the pre-mRNA targets

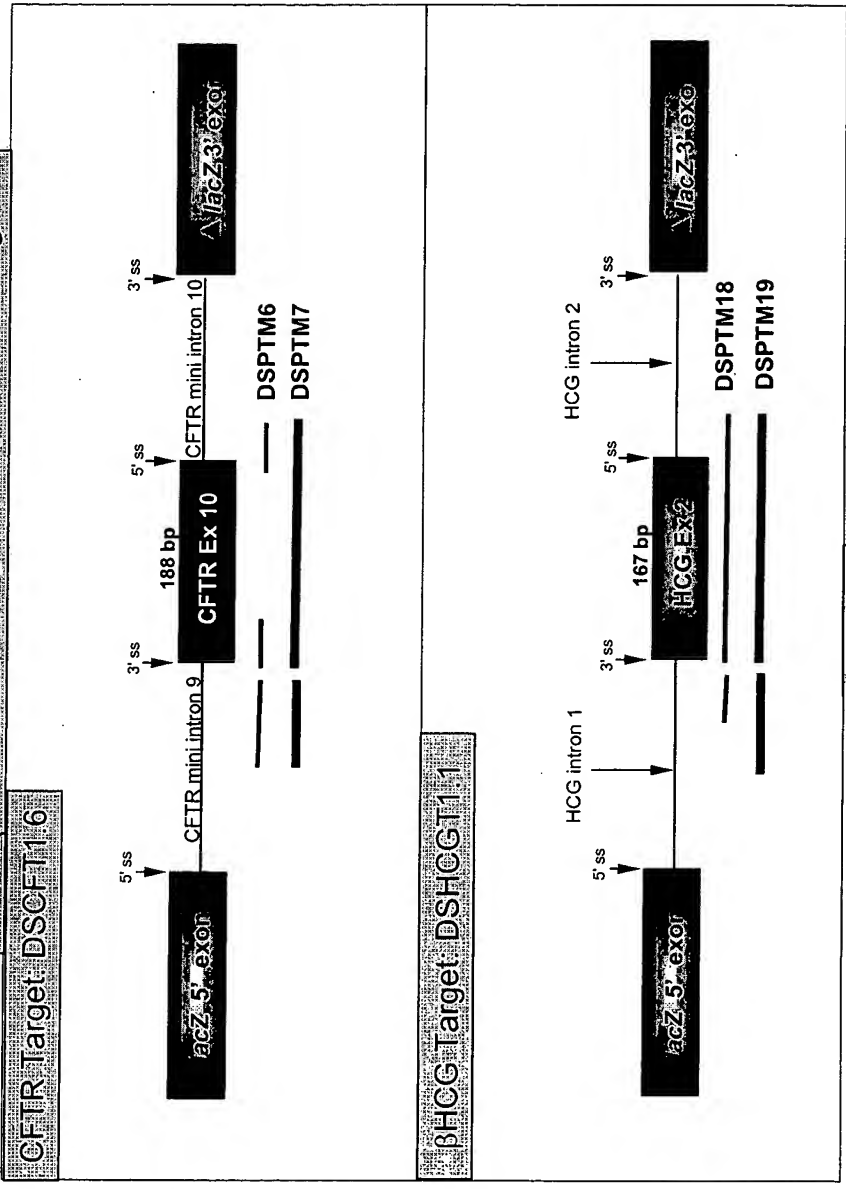
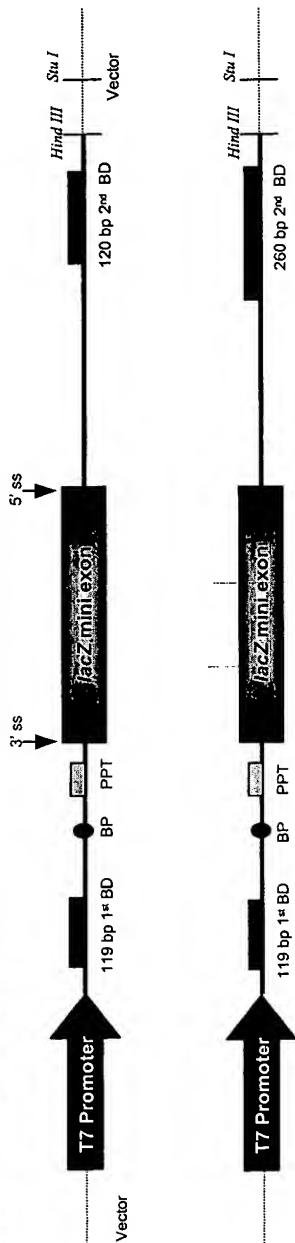


Figure 2

Schematic diagrams of double trans-splicing PTMs

DSPTM6 & 7 (CFIR Targeted)



DSPTM18 & 19 (βHCG Targeted)

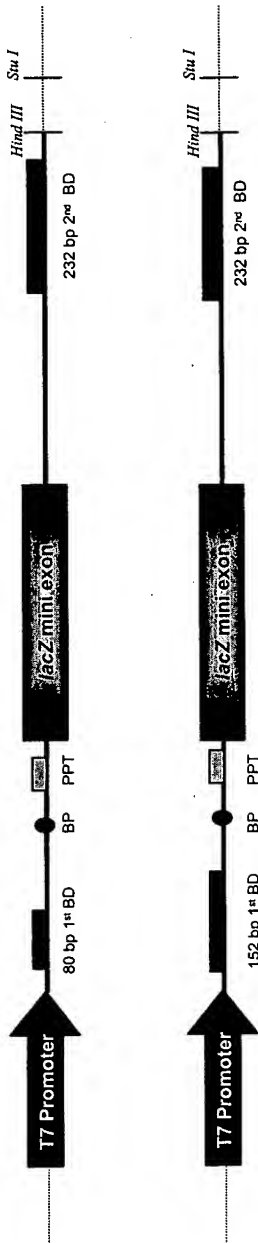
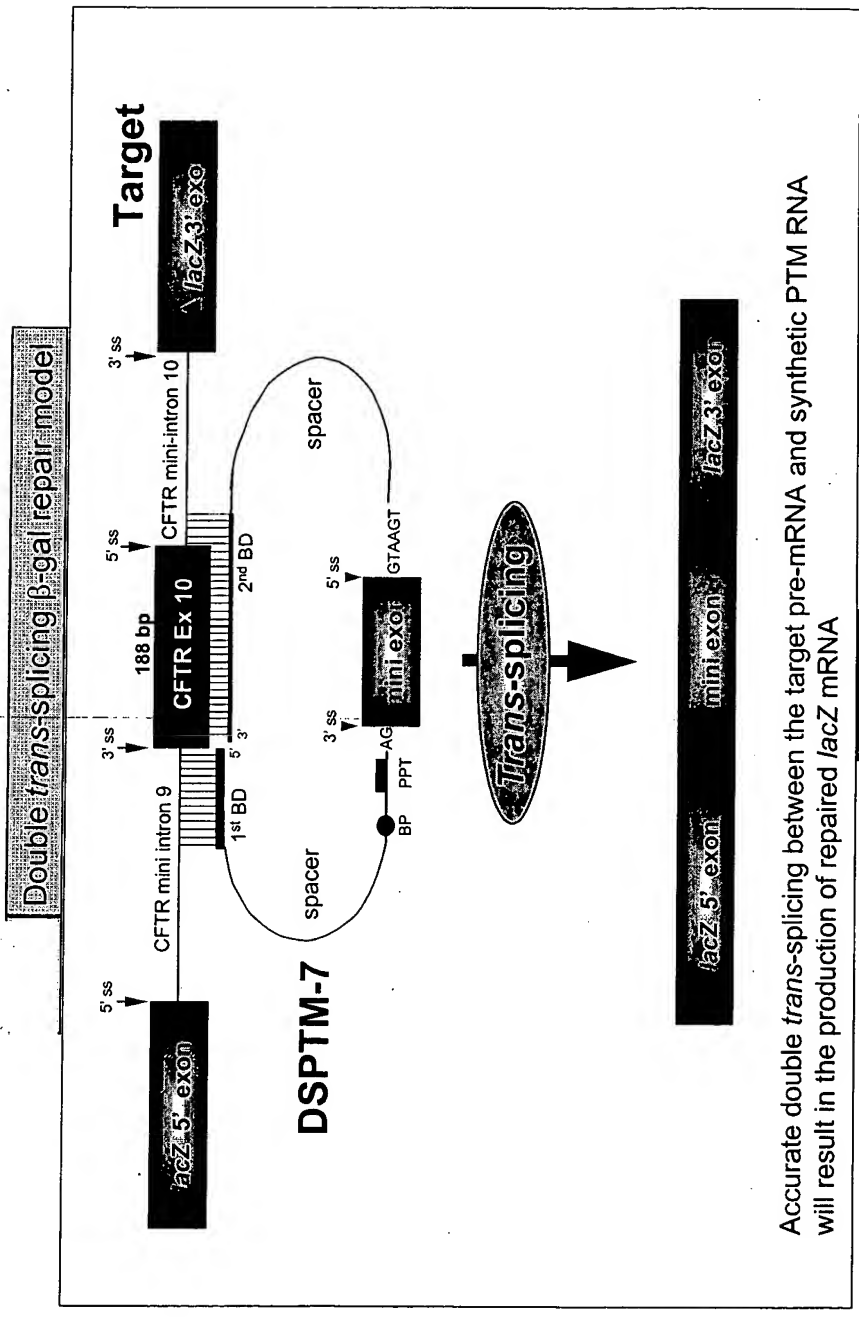


Figure 3

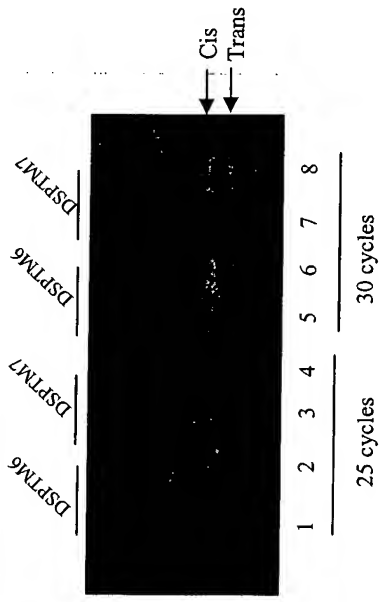
Figure 5



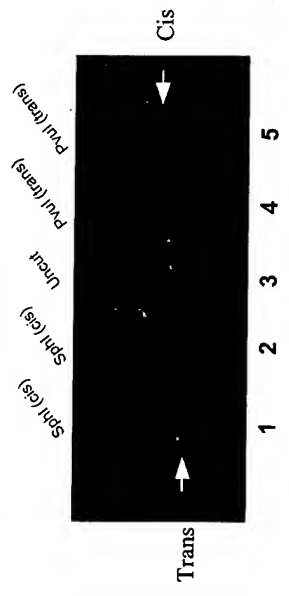
Accurate double trans-splicing between the target pre-mRNA and synthetic PTM RNA will result in the production of repaired lacZ mRNA

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in 293T cells

2nd PCR Amplification



Diagnostic Test



DSPTM6 and 7 (CFTR targeted)

Methods

Transfect 293T cells with DSPTM6 and DSPTM7 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycles, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/1h

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F+Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp.

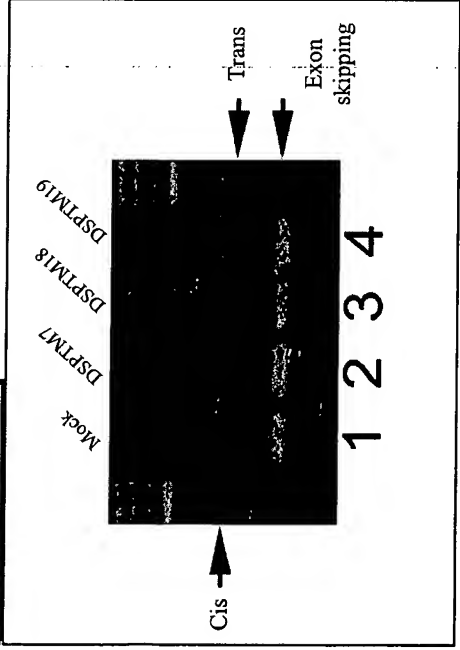
Diagnostic test: Digest PCR product with *Pvu* I (*trans*-specific) and with *Sph* I (*cis*-specific) at 37°C for 2-3 hr

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6A

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in stable cells

2nd PCR Amplification



DSPTM18 and 19 (HCG targeted)

Methods

Transfect DSHCGT1.1 stable cells with DSPTM7, DSPTM18 and DSPTM19 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycles, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F + Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6B

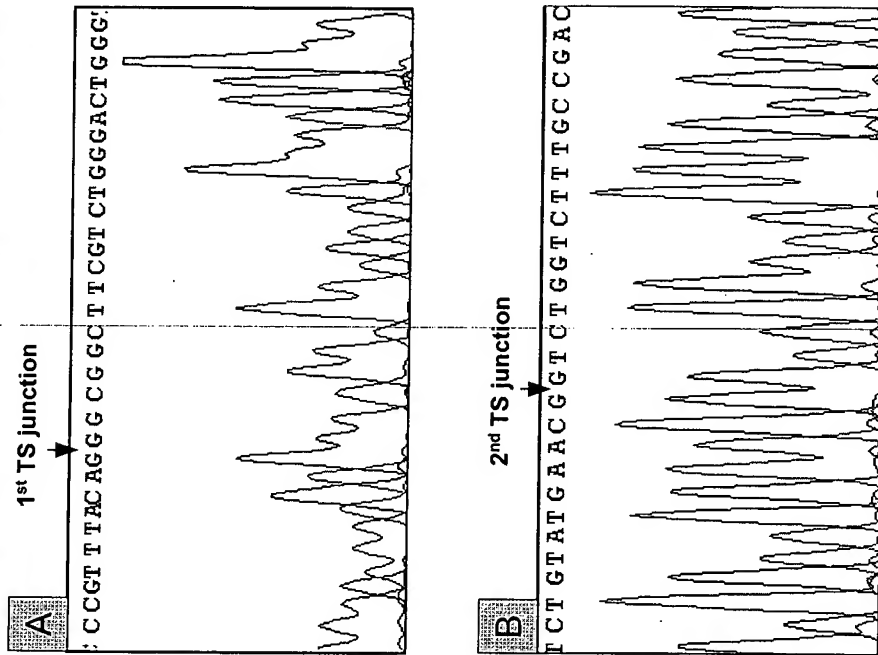
Accuracy of double *trans*-splicing of synthetic PTM RNA in 293T cells

Figure 6C

Restoration of β -gal function through RNA transfection in 293T cells
(Proof-of-concept for SMaRT RNA Therapeutics!!)
Synthetic RNA, Double *trans*-splicing

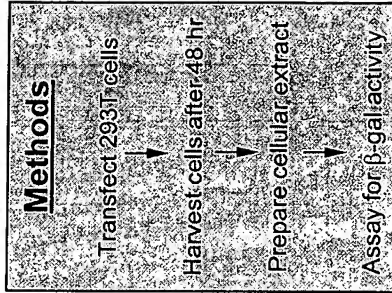
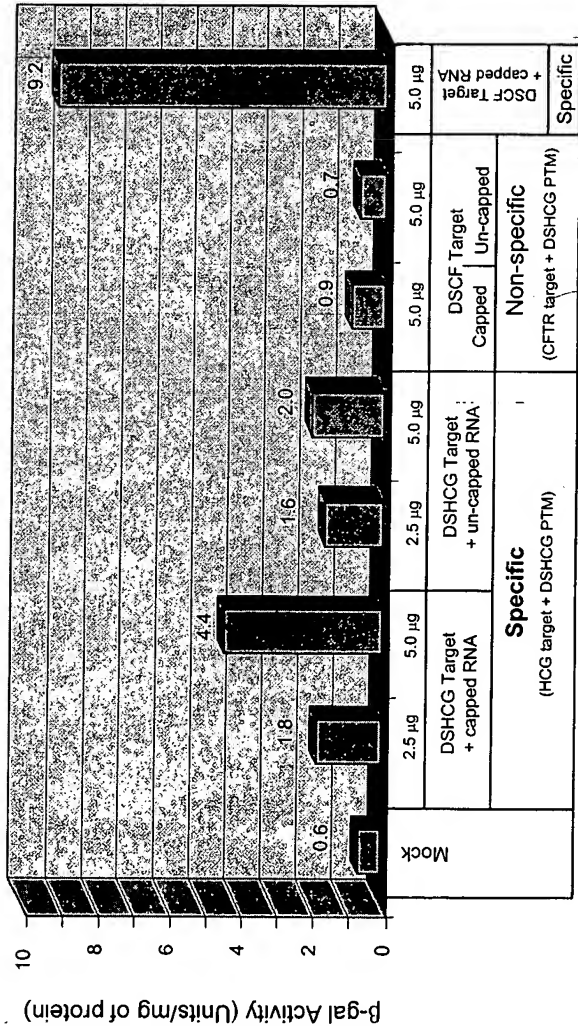


Figure 7